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(54) Title: GENE ASSOCIATED WITH DISEASE RESISTANCE IN PLANTS

(57) Abstract

A gene encoding a novel mitogen–activated protein ("MAP") kinase has been identified in rice, isolated and cloned. Expression of the gene is induced in response to infection with the blast pathogen, M.grisea. The gene has utility in conferring disease resistance to plants, particularly monocotyledonous plants, such as rice, wheat, maize, barley and asparagus. Vectors containing the novel gene and transformed plant cells, plants and seeds are also disclosed.

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GENE ASSOCIATED WITH DISEASE RESISTANCE IN PLANTS

Background of the Invention

Field of the Invention

Bacterial, fungal and viral infections of plants grown for food and fiber cause substantial economic losses to farmers and consumers. For example, rice blast is the most economically devastating disease of cultivated rice, caused by the filamentous fungus *Magnaporthe grisea* (Ou, 1985). (A bibliography is provided at the end of the written description.) It occurs in most rice growing areas worldwide, costs farmers $5 billion annually (Moffat, 1994). The disease reduces rice yield significantly, particularly in the temperate flooded and tropical upland rice ecosystem. The use of resistant cultivars is the most economical and effective method of controlling the disease. With the advent of transgenic plant technology, it is possible to identify natural host defense mechanisms and to transfer genes associated with these mechanisms to or control expression of such genes in commercial cultivars. It is hoped that expression of such genes will confer disease resistance to the transgenic plants.

Background Art

Over the last decades, much has been learned about the genetics of resistance to the blast fungus. While the molecular mechanism of host defenses to this pathogen is mostly unknown, blast fungus is believed to infect rice plants in a manner typical of other foliar pathogens. Infection by *M.grisea* is initiated when a conidium lands on a leaf surface. In a drop of water, a conidium produces a germ tube that grows and differentiates a specialized infection structure called an appressorium that adheres tightly to the plant surface (Bourett and Howard, 1990). The specialized cell generates enormous turgor pressure that is used to penetrate the underlying plant surface (Howard, 1994). The penetration into plant cells by pathogen invasion may damage the cell structure and activate genes responsive to wounding.

In plants, two mitogen-activated protein ("MAP") kinases involved in defense response to wounding have been identified (Usami et al., 1995; Bogre et al., 1997). Usami et al., (1995) reported a MAP kinase that is induced by wounding leaves from a variety of plant species including dicotyledonous and monocotyledonous plants. Another MAP kinase in alfalfa, p44MMK4, was activated by wounding. After wounding, the activity of p44MMK4 rose within 1 minute but decreased to basal levels within 30 minutes. It has been demonstrated that a MAP kinase, *PMK1*, plays a role in appresorrium formation and infectious growth in rice blast fungus *M.grisea* (Xu and Hamer, 1996).

The MAP kinase signaling cascade is one of the major pathways involved in transducing extracellular stimuli into intracellular responses in mammals and yeasts (Shyy and Chien, 1997, Gabay et al., 1997, Samejima et al., 1997). MAP kinase is a specific class of serine/threonine protein kinases and has been implicated in a wide variety of physiological processes, such as cell growth, differentiation, oncogenesis and response to environmental stresses (Herskowitz, 1995, Cohen, 1997). In mammals, MAP kinases or extracellular signal regulated kinases ("ERKs") were originally identified as transducers of mitogens (substances that induce proliferation). Later, MAP kinases were also shown to be involved with signaling hormones, neurotransmitters and signals for differentiation(Marshall, 1994). At present, MAP kinase pathways are best understood in yeast and animals and several distinct MAP kinase pathways have been identified (Ruis and Schuller, 1995). The basic module of a MAP kinase cascade is a specific set of three functionally interlinked kinases. The activation of MAP kinases is brought about by upstream (i.e. earlier in the reaction sequence) kinases through phosphorylation of the conserved threonine and tyrosine residues that are located close to kinase domain VIII in all MAP kinases (Marshall, 1994; Hirt, 1997). These dual-specificity MAP kinase kinases (MAPKKs) can only catalyse the activation of specific MAP kinase and can not substitute for each other. The MAPKKs are themselves activated by phosphorylation through upstream kinases that either belong to the class of MAPKK kinases (MAKKKs), or are raf and mos proteins (Marshall, 1994; Hirt, 1997).

In plants, several genes encoding MAP kinases have been identified from alfalfa (Jonak et al., 1993; 1995), *Arabidopsis* (Mizoguchi et al., 1994), pea (Stafstrom et al., 1993), petunia (Decroocq-Ferrant et al., 1995), tobacco (Wilson et al., 1993) and parsley (Ligterink et al., 1997). Similar to mammalian kinases, AtMAPK1 and AtMAPK2 are shown to be involved in cell proliferation (Jonak et al., 1993, Mizoguchi et al., 1994). Several stress-induced MAP kinases have also been identified in plants which are responsive to cold, heat, wounding, drought and mechanical stresses (Bogre et al., 1997, Jonak et al., 1996; Seo et al., 1995, Ligterink et al., 1997; Zhang and Klessig, 1997). The 48 kD MAP kinase, ERMK, is rapidly activated upon high-affinity binding of a fungal elicitor to a plasma membrane receptor in parsley cells (Ligterink et al., 1997). The activated ERMK is translocated into the nucleus where it may be involved in the transcriptional activation of defense genes. Recently, a MAP kinase, p48 SIP, is identified to be activated in tobacco cells by salicylic acid (SA) treatment which is an endogenous signal for the activation of several plant defense response (Zhang and Klessig, 1997).

These studies suggest that MAP kinases are an important component in the signal transduction pathway of plant defense to pathogen infection. Ligterink et al. (1997) and Zhang and Klessig, (1997) have found a elicitor-responsive MAP kinase in parsley suspension cells and a SA-activated MAP kinase in tobacco suspension cells respectively. However, no evidence was found that MAP kinase is activated by natural pathogen infection in plant species. Accordingly, a need exists for the identification of MAP kinase genes associated

with such defense mechanisms and means for expressing such genes in host plants (or regulating their expression) to confer disease resistance.

Summary of the Invention

In accordance with this invention, a novel MAP kinase gene and protein that it encodes have been discovered. Based on sequence analysis, this novel gene is a new member of the MAP kinase gene family which encodes a 519 amino acid 59 kD protein. It is designated as BIMK1 for blast induced MAP kinase. BIMK1 was strongly induced by rice blast fungus *M.grisea* and is postulated to be involved in the defense response of rice to blast infection.

In one aspect, the invention relates to the deoxyribonucleic acid ("DNA") that comprises the novel MAP kinase gene, its messenger ribonucleic acid ("mRNA) transcript and the protein that it encodes. In related aspects, the invention involves expression vectors that contain the novel gene operably linked to a plant active promoter and to plant cells and plants that have been transformed with such vectors.

In a further aspect, the invention concerns a method for conferring disease resistance in plants, particularly monocot plants such as rice, wheat, maize, barley and asparagus, which comprises genetically modifying the plant to effect expression of the novel MAP kinase gene.

Brief Description of the Drawings

Figure 1 is an autoradiogram of a Southern hybridization analysis of restriction enzyme digested rice genomic DNA using labeled BIMK1 cDNA as a probe.

Figure 2 is an autoradiogram of a Northern analysis of total RNA (50 mg) isolated from rice leaf tissue at different time points after inoculation with *M.grisea* using labeled BIMK1 cDNA as a probe.

Detailed Description of the Invention

The gene encoding a MAP kinase, identified as BIMK1 has been identified for rice, cloned and sequenced. The sequence of the full-length clone, including 5' and 3' untranslated regions, is provided in SEQ ID NO:1. The region from nucleotide 13 through nucleotide 1569 encodes the 519 amino acid 59kD protein whose sequence is shown in SEQ ID NO:2. The BIMK1 gene was isolated from rice infected with the rice blast pathogen, *Magnaporthe grisea*.

The invention provides an isolated DNA having substantially the sequence spanning nucleotides 13 through 1569 of SEQ ID NO:1. The invention further provides isolated mRNA complementary to the deoxyribonucleic acid having substantially the sequence spanning nucleotides 13 through 1569 of SEQ ID NO:1. The invention also provides an isolated protein having substantially the sequence shown in SEQ ID NO:2. "Isolated" as used herein, means that the nucleic acid or protein is in an environment different from its natural environment. For example, it may be cloned in a cloning or expression vector, it may reside in a bacterial cell, it may be associated with other means for transformation of plants or plant cells or it may

reside in a plant with which it is not naturally associated. As used herein, the term "substantially the sequence" means a sequence that is predominantly that of the identified sequence, provided that the nucleic acid or protein retains the kinase functions of the native molecule. Thus, conservative substitutions, deletions and additions that do not significantly reduce the function of the protein are contemplated.

Probes, primers, antisense molecules and other nucleic acid molecules that are complementary to regions of the BIMK1 gene will be useful for its amplification and analysis, regulation of its expression and the like. Accordingly, the invention provides DNA or RNA molecules that are capable of hybridizing to the DNA molecules described above (or their complements) under stringent hybridization conditions. Such conditions are well known in the art and include those conditions under which stable hybrids will form when there is at least about 75%, preferably at least about 80%, most preferably at least about 90%-100% homology between the DNA or RNA molecule and the corresponding region of the target DNA.

The DNA can be incorporated in plant or bacterial cells using conventional recombinant DNA technologies. Generally, such techniques involve inserting the DNA into an expression vector which contains the necessary elements for the transcription and translation of the inserted protein coding sequences and one or more marker sequences to facilitate selection of transformed cells or plants.

A number of plant-active promoters are known in the art and may be used to effect expression of the nucleic acid sequences disclosed herein. Suitable

promoters include, for example, the nos promotor, the small subunit chlorophyll A/B binding polypeptide, the 35S promotor of cauliflower mosaic virus, and promoters naturally associated with MAP kinase genes, such as BIMK1 in plants. SEQ ID NO: 6 provides the sequence of the 5' untranslated region upstream of the BIMK1 coding sequence. This region contains the putative promoter for this gene. SEQ ID NO: 6 overlaps the 5' end of the BIMK1 coding region, the ATG start codon appearing at position 1378-80. A "TATA" box appears at positions 1302-1306 of the sequence. In addition to directing expression of the MAP kinase DNA described herein, this promoter has general utility as a plant-active promoter, particularly for effecting expression of transgenes in monocotylodonous plants, such as rice.

Once the isolated DNA of the present invention has been cloned into an expression vector, it may be introduced into a plant cell using conventional transformation procedures. The term "plant cell" is intended to encompass any cell derived from a plant including undifferentiated tissues such as callus and suspension cultures, as well as plant seeds, pollen or plant embryos. Plant tissues suitable transformation include leaf tissues, root tissues, meristems, protoplasts, hypocotyls, cotyledons, scutellum, shoot apex, root, immature embryo, pollen, and anther.

One technique for transforming plants is by contacting tissue of such plants with an inoculum of a bacterium transformed with a vector comprising DNA in accordance with the present invention. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48

to 72 hours on regeneration medium without antibiotics at 25-28° C.

Bacteria from the genus *Agrobacterium* can be utilized advantageously to transform plant cells. Suitable species of such bacteria include *Agrobacterium tumefaciens* and *Agrobacterium rhizogens*. *Agrobacterium tumefaciens* (*e.g.*, strains LBA4404 or EHA105) is particularly useful due to its well-known ability to transform plants.

Another approach to transforming plant cells with the nucleic acid of this invention involves propelling inert or biologically active particles into plant cells. This technique is disclosed in U.S. Pat. Nos. 4,945,050, 5,036,006 and 5,100,792 all to Sanford et. al., which are hereby incorporated by reference. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector comprising the isolated DNA of this invention. Biologically active particles (*e.g.*, dried yeast cells, dried bacterium or a bacteriophage, each containing DNA sought to be introduced) can also be propelled into a plant cell tissue.

Another method of transforming plant cells is the electroporation method. This method involves mixing the protoplasts and the desired DNA and forming holes in the cell membranes by electric pulse so as to introduce the DNA into the cells, thereby transforming the cells. This method currently has high

reproducibility and various genes have been introduced into monocotyledons, especially rice plants by this method (Toriyama et. al., 1988, Shimamoto et al., 1989 and Rhodes et al., 1988).

Similar to the electroporation method is a method in which the desired gene and protoplasts are mixed and the mixture is treated with polyethylene glycol ("PEG"), thereby introducing the gene into the protoplasts. This method is different from the electroporation method in that PEG is used instead of an electric pulse (Zhang W. et. al., 1988, Datta et al., 1990 and Christou et al., 1991).

Other methods include 1) culturing seeds or embryos with nucleic acids (Topfer R. et al., 1989, Ledoux et al., 1974) 2) treatment of pollen tube, (Luo et al., 1988) 3) liposome method (Caboche, 1990 and Gad et al., 1990) and 4) the microinjection method (Neuhaus G. et al., 1987).

Known methods for regenerating plants from transformed plant cells may be used in preparing transgenic plants of the present invention. Generally, explants, callus tissues or suspension cultures can be exposed to the appropriate chemical environment (e.g., cytokinin and auxin) so the newly grown cells can differentiate and give rise to embryos which then regenerate into roots and shoots.

The isolated DNA of the present invention is believed to be useful in enhancing resistance to disease-causing pathogens in both monocotyledonous plants ("monocots"), and dicotyledonous plants ("dicots"). It is preferred for use with commercially important monocots, such as rice, wheat, barley, maize and asparagus.

In plants in which the BIMK1 gene naturally resides, enhanced disease resistance may be achieved by controlling expression of the endogenous gene, rather than transforming the plant with a vector containing the gene. Such control may be achieved, for example, by modifying or replacing endogenous promoters, enhancers or other control signals that regulate expression of the gene, for example, to achieve enhanced expression or programmed expression.

The predicted protein sequence of BIMK1 carries all 11 conserved domains for the catalytic function of serine/threonine protein kinase. The expression of BIMK1 was rapidly induced as early as 4 hours after inoculation with *M.grisea*, evincing the involvement of BIMK1 in the defense response to the blast fungus.

Several stress-induced MAP kinases have been identified in dicots. As shown in Table 1 below, the protein sequences of these genes showed 70-75% homology. For example, Parsley ERMK and tobacco SIMK have 74.4% protein identity. However, as shown in Table 1, BIMK1 only has about 50% identity with these two stress-related MAP kinases isolated from dicot plants. This suggests the divergence of MAP kinases in monocot and dicot plant species. In addition to sequence differences, BIMK1 is about 500 bp longer than all cloned MAP kinase genes. The 3' region of the gene contains a domain similar with ADH genes in animals. The function of this domain in the defense response to blast infection is unknown.

The invention is further illustrated by the following examples, which are not intended to be limiting.

EXAMPLES

Materials and Methods

Rice plants and blast inoculation

The resistant isogenic line C101A51 carrying the Pi-2 gene and the susceptible cultivar C039 were used in the experiment. Three week-old rice plants were inoculated with a Philippine isolate PO6-6 of *M.grisea*. After inoculation, plants were kept in dark in a dew chamber for 24 hours at 26° C. Then, inoculated plants were move into a growth chamber in 10 hours light with 14 hours dark at 25-26° C for 7 days. Leaf tissue was harvested from both cultivars at 0, 4, 8, 12, 24, 48. 72 hours after inoculation.

RNA isolation, cDNA svnthesis and RT-PCR

RNeasy mini kit (Qiagen, Germeny) was used to isolate total RNA from 150-200 mg rice leaf tissue. Poly(A)+ RNA fractionated from total RNA using Qiagen Oligotex Spin Column was used as a template in a reverse transcriptase-mediated polymerase chain reaction (RT-PCR). Two primers, CF9-RT and CF9-Rev, were designed based on the DNA sequence of the cloned gene Cf-9, a tomato resistance gene to the leaf mould fungus *Cladosporium fulvum* (Jones et al., 1994). The primer sequence of CF9-RT is 5'-AAAAGCACAAGTTGCTGC-3' (SEQ ID NO:3) which is the DNA sequence 217-235 bp after the start codon. The sequence of CF9-Rev is 5'TAACGTCTATCGACTTCT-3' (SEQ. ID NO:4) which is the reverse strand sequence of Cf-9 from 1408 to 1426 bp after the start codon. RT-PCR was conducted following protocols provided by the manufacturer (GIBCO-BRL,

Life-Technology, USA). The amplified cDNAs were then separated in 1.2% agarose gel.

Cloning and DNA sequencing

Specific bands were cloned into pGEM-T vector (Promega, USA). Clones were sequenced using the ABI PRISM 377 DNA sequencer (Perkin-Elmer, CA, USA). The sequence was analyzed with softwares DNAstar and Sequencher 3.0.

BAC library screening and subcloning

Protocols for BAC filter preparation and screening were as described Wang et al. (1995). Hybridization and washing conditions were the same as described in Hoheisel et al., (1993).

Southern hybridization

Rice genomic DNA was isolated as described by Dellporta et al. (1984). DNA was digested with restriction enzymes and separated in 0.8% agarose gel, and then transferred onto Hybond-N+ membrane (Amersham, UK). Probes were labeled using megaprimer labelling kit (Amersham, UK). Rapid hybridization solution (Clonetech, USA) was used.

Northern hybridization

Total RNA used in the Northern blot analysis was isolated using a Trizol total RNA isolation reagent (GIBCO-BRL, Life-Technology, USA). Fifty micrograms of total RNA per lane was separated in 1.0% agarose gel and transferred onto Hybond-N+ membrane (Amersham, UK) using NorthernMax kit (Ambion, USA) following the manufacturer's instruction. Northern hybridization was carried out same as Southern hybridization described above.

Example 1

Isolation of a cDNA fragment induced after blast infection

Total RNA was isolated from leaf tissue inoculated with isolate PO6-6 8 hours after inoculation. Purified mRNA was used as template in the first strand cDNA synthesis. When primers CF9-RT and CF9-Rev were used in RT-PCR, four bands were amplified in both ClOlA51 (compatible) and C039 (incompatible) post-inoculation (data not shown). These cDNA fragments were then cloned into the pGEM-T vector. Clones with different insert sizes were sequenced. A database search revealed that the clone with 350 bp insert is highly homologous to mammalian and yeast MAP kinases.

Example 2

Isolation of genomic clones from rice BAC library

To clone the full-length genomic fragment of this gene, a rice BAC library of cultivar IR64 (Yang et al., 1997) was screened using the 350 bp cDNA fragment

described in Example 1 as a probe. Four positive BAC clones (3-07, 17-H21, 43-H15 and 43-F5) were identified from the whole BAC library. The miniprepared DNA of the three BAC clones was digested with 3 different enzymes to check if they are overlapping clones in a chromosomal region. Based on the restriction patterns, it was found that these three clones were overlapping clones. Thus, one BAC clone (3-07) was chosen and subcloned into pBluescript-SK (Strategene, USA). The recombinant clone which hybridized with the 350 bp cDNA fragment (M1, 4.5 kb) was identified and used for sequencing. Based on a comparison with known MAP kinase genes, it was found that it contains the 5' region of the gene including the putative promoter and part of coding region (about 400 bp).

Example 3

Isolation of a full-length cDNA using RT-PCR

To isolate a full length cDNA from rice, a primer containing sequence spanning the start codon ATG(5'-AACACAGTGGAAATGGAGTTCTTCA-3') SEQ ID NO:5 was designed based on the genomic DNA sequence. RT-PCR was performed using this primer and a oligo-dT primer (Life-Technologies, USA). From the cDNA prepared from the infected leaves of C101A51 (8 hours after inoculation), a 2.0 kb PCR product was obtained. This PCR product was cloned into pGEM-T vector and sequenced. The sequence is shown in SEQ ID NO:1. It contains a 1557 bp open reading frame corresponding to 519 amino acids (SEQ ID NO:2). This gene was designated BIMK1 for blast induced MAP kinase. This amino acid sequence was compared to the sequence of

several MAP kinases isolated from a variety of organisms. As shown in Table 1, the sequences are significantly homologous. In section A of the Table, multiple alignment of the deduced amino acid sequence (N-terminal) of BIMK1 with other members of MAP kinases from other organisms is shown. The amino acid sequence of BIMK1 is compared to that of MsERK (Duerr et al., 1993) from *Medicago sativa*, WIPK (Seo et al., 1995) from tobacco, ATMPK (Mizuguchi et al., 1994) from *Arabidopsis*, ERK2 (Owaki el al., 1992) from human, ERM (Ligterink et al., 1997) from parsley. Bold type represents amino acid residues that match the EIMK1. Gaps were induced to maximize alignment. The conserved TXY (in BIMK1, "X" is an aspartic acid while in most MAP kinase it is a glutamic acid) phosphorylation motif for MAP kinase is indicated by asterix. The 11 MAP kinase subdomains are labeled in Roman numerals (Hanks et al., 1988). The *M.grisea* BIMK1 gene contains all 11 highly conserved subdomains which are present in all known MAP kinases in mammals and plants. Interestingly, BIMK1 also contains 50 amino acids homologous to mammalian alcohol dehydrogenase (ADH) in its C-terminal. Section B of the Table shows multiple alignment of the deduced amino acid sequence (C-terminal) of BIMK1 with other ADH genes in animals and plants. ADH is present in many organisms that metabolize ethanol, including human, in an oxidoreductase reaction with NAD+/NADH as an essential co-factor.

Example 4

BIMK1 is conserved in rice genome and mapped to a region clustering blast resistance genes

DNAs of C101A51 and C039 were digested the restriction enzymes BamHI, EcoRI and HindIII. Southern hybridization was carried out as described in the section of Materials and Methods using the cDNA fragment of BIMK1 as probe. No polymorphism was detected between resistant and susceptible lines for three enzymes (Figure 1). Similar results have been obtained using DNAs of 4 other cultivars (data not shown). These result indicated that BIMK1 is conserved among rice cultivars. BIMK1 has been mapped on rice chromosome 12 between makers RG341 and RG574, a region clusterring rice blast resistance genes Pi-4(t) and Pi-6(t).

Example 5

BIMKI was induced bv rice blast fungus

Total RNA was isolated from rice leaf tissue collected at different timepoints after inoculation. The blot was hybridized using BIMK1 cDNA fragment as probe labelled with 32p. It was found that BIMK1 was highly induced as early as 4 hours after inoculation. The expression of the gene BIMK1 was reduced 24 hours after inoculation (Figure 2). The induction level of BIMK1 in both resistant (C101A51) and susceptible (C039) lines was very similar (Figure 2). Since C101A51 and Co39 have the same genetic background except C101A51 carries a rice blast resistance gene, Pi-2, it is suggested that BIMK1 was induced independently from Pi-2 and is involved in a general defense pathway to blast.

Table 1

(A)

```
                                                                                I                   II
BIMK1  ---------------- ---------------- ----------MEFFT EYGEASQ-YQIQ-EV IGKGSYGVVAAAVDT RTGERVAIKKINDVF 48
MsERK1 MEGGGAPPADTVMSD AAPAPPQMGIENIPA VLSHGGRFIQYNIFG NIFEVTAKYKPPIMP IGKGAYGIVCSAHNS ETNEHVAVKKIANAF 90
WIPK   ------------MAD ANMGAGGGQFPDFPS VLTHGGQYVQFDIFG NFFEITTKYRPPIMP IGRGAYGIVCSVLNT ELNEMVAVKKIANAF 78
ATMPK1 ---------------- ------MATLVDPPN GIRNEGK-HYFSMWQ TLFEIDTKYMP-IKP IGRGAYGVVCSSVNS DTNEKVAIKKIHNVY 67
ERK2   ---------------- ------------MA AAAAAGP---EMVRG QVFDVGPRYTN-LSY IGEGAYGMVCSAYDN LNKVRVAIKKIS-PF 57
ERM    ---------------- -MANPGDGQYTDFPA IQTHGGQFIQYNIFG NLFQVTKKYRPPIMP IGRGAYGIVCSIMNT ETNEMVAVKKIANAF 74

                  III       IV                                V                                       VI
BIMK1  EHVSDATRILREIKL LRLLRHPDIAEIKHI MLPPSRREFQDIYVV FELMESDLHQVIRAN DDLTPEHYQFFLYQL LRALKYIHAANVFHR 138
MsERK1 DNKIDAKRTLREIKL LRHMDHENVVAIRDI VPPPQREVFNDVYIA YELMDTDLHQIIRSN QALSEEHCQYFLYQI LRGLKYIHSANVLHR 180
WIPK   DIYMDAKRTLREIKL LRHLDHENVIGLRDV IPPPLRREFSDVYIA TELMDTDLHQIIRSN QGLSEDHCQYFMYQL LRGLKYIHSANVLHR 168
ATMPK1 ENRIDALRTLRELKL LRHLRHENVIALKDV MMPIHKMSFKDVYLV YELMDTDLHQIIKSS QRLSNDHCQYFLFQL LRGLKYIHSANILHR 157
ERK2   EHQTYCQRTLREIKI LLRFRHENIIGINDI IRAPTIEQMKDVYIV QDLMETDLYKLLKT- QHLSNDHICYFLYQI LRGLKYIHSANVLHR 146
ERM    DNYMDAKRTLREIKI LRHLDHENVIAITDV IPPPLRREFTDVYIA TELMDTDLHQIIRSN QGLSEEHCQYFLYQL LRGLKYIHSANIIHR 164

                       VII                     * * VIII                      IX
BIMK1  DLKPKNILANSDCKL KICDFGLARASFNDA PSAIFWTDYVATRWY RAPEIMWLIFSKYTP AIDIWSIGCIFAELL TGRPLFPGKNVVHQL 228
MsERK1 DLKPSNLLLNANCDL KICDFGLARVTSET- ---DFMTEYVVTRWY RAPELL-LNSSDYTA AIDVWSVGCIFMELM DRKPLFPGRDHVHQL 265
WIPK   DLKPSNLLVNANCDL KICDFGLARPNIEN- ---ENMTEYVVTRWY RAPELL-LNSSDYTA AIDVWSVGCIFMELM NRKPLFGGKDHVHQI 253
ATMPK1 DLKPGNLLVNANCDL KICDFGLARASNTKG ---QFMTEYVVTRWY RAPELL-LCCDNYGT SIDVWSVGCIFAELL GRKPIFQGTECLNQL 243
ERK2   DLKPSNLLLNTTCDL KICDFGLARVADPDH DHTGFLTEYVATRWY RAPEIM-LNSKGYTK SIDIWSVGCILAEML SNRPIFPGKHYLDQL 235
ERM    DLKPSNILLNANCDL KICDFGLARHNTDDE ----FMTEYVVTRWY RAPELL-LNSSDYTV AIDIWSVGCIYMELM NRKPLFPGKDHVHQM 249

             X                                                                 XI
BIMK1  DIITDLLGTPSSETL SRIRNEKARRYLSTM RKKHAVPFSQKFRNT DPLALRLLERLLAFD PKDRPSAEEALADPY FASLANVEREPSRHP 318
MsERK1 RLLMELIGTPSEDDL GFL-NENAKRYIRQL PPYRRQSFQEKFPHV HPEAIDLVEKMLTFD PRKRITVEDALAHPY LTSLHDISDEP--VC 352
WIPK   RLLTELLGTPTEADL GFLQNEDAKRYIRQL PQHPRQQLAEVFPHV NPLAIDLVDKMLTFD PTRRITVEEALDHPY LAKLHDAGDEP--IC 341
ATMPK1 KLIVNIIGSQREEDL EFIVNPKAKRYIRSL PYSPGMSLSRLYPCA HVLAIDLLQKMLVFD PSKRISASEALQHPY MAPLYDPNANP--PA 331
ERK2   NHILGILGSPSQEDL NCIINLKARNYLLSL PHKNKVPWNRLFPNA DSKALDLLDKMLTFN PHKRIEVEQALAHPY LEQYYDPSDEP--IA 323
ERM    RLLTELLGSPTEADL GFVRNEDAKRFILQL PRHPRQPLRQLYPQV HPLAIDLIDKMLTFD PSKRITVEEALAHPY LARLHDIADEP--IC 237

BIMK1  ISKLEFEFERRKLTK DDVRELIYREILEYH PQMLQEYMKG 358(519)
MsERK1 MTPFSFDFEQHALTE EQMKELIYREALAFN PEYQQ      387
WIPK   PVPFSFDFEQQGIGE EQIKDMIYQEALSLN PEYA       375
ATMPK1 QVPIDLDVDED-LRE EMIREMIWNEMLHYH PQASTLNTEL 370
ERK2   EAPFKFDMELDDLPK EKLKELIFEETARFQ PGYRS      358
ERM    TKPFSFEFETAHLGE EQIKDMIYQEALAFN PDCA       371
```

Table 1 (continued)

(B)

```
BIMK1-rice  445 SAGQNGVTSTDLSSRSYLKSAS-ISASKCVAVKDNKEPEDDYISEEM-EGSVDGLFEQVF-RMQFLV 509
ADH-rabbit  214 AAGASRIIAVDINKDKFPK-AKEVGATECINPQDYKKPIQEVIQE-ISDGGVDFSFE-VIGRLDTVV 277
ADH-horse   212 AAGAARIIGVDINKDKFAK-AKEVGATECVNPQDYKKPIQEVLTE-MSNGGVDFSFE-VIGRLDTMV 275
ADH-human   161 AAGAARIIAVDINKDKFAK-AKELGATECINPQDYKKPIQEVLKE-MTDGGVDFSFE-VIGRLDTMM 224
```

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Institute for Molecular Agrobiology (except for US)

He, Chaozu (for US)
Wang, Guo-Liang (for US)

(ii) TITLE OF INVENTION: Gene Associated with Disease Resistance in Plants

(iii) NUMBER OF SEQUENCES: 6

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1957 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Oryza sativa
(B) STRAIN: C101A51

(vii) IMMEDIATE SOURCE:
(B) CLONE: BIMK1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AACACAGTGG AAATGGAGTT CTTCACTGAG TATGGAGAAG CAAGCCAGTA CCAGATCCAG 60

GAAGTCATTG GCAAAGGAAG TTATGGAGTA GTTGCTGCTG CAGTAGATAC CCGCACGGGT 120

GAGCGGGTTG CGATCAAGAA GATCAATGAT GTGTTTGAGC ATGTATCAGA CGCTACGCGC 180

ATATTGCGTG AGATCAAGCT CCTTCGTCTG CTCCGTCACC CAGACATAGC TGAGATCAAA 240

CACATTATGC TTCCCCCTTC TCGAAGGGAG TTCCAAGATA TTTATGTTGT TTTTGAGCTC 300

ATGGAATCAG ATCTCCATCA AGTCATCAGA GCGAACGATG ACCTCACCCC GGAGCACTAC 360

CAGTTTTTCC TGTACCAACT TCTTCGTGCT CTCAAGTACA TCCATGCAGC TAATGTATTT 420

CATCGCGATC TAAAGCCCAA GAATATACTG GCAAACTCAG ACTGCAAATT GAAAATATGT 480

GATTTCGGAC TTGCCCGAGC ATCATTCAAT GATGCCCCTT CAGCAATATT TTGGACGGAT 540

TATGTTGCAA CGAGGTGGTA CCGAGCACCT GAATTATGTG GCTCATTTTT CTCCAAATAC 600

ACTCCTGCAA TTGATATTTG GAGTATTGGG TGCATATTTG CTGAACTTCT CACTGGGAGA 660

CCACTATTTC CTGGGAAGAA TGTTGTGCAC CAATTAGATA TTATAACAGA TCTTCTTGGA 720

ACTCCATCAT CAGAAACCTT ATCCAGGATT CGAAATGAGA AGGCCAGGAG ATACTTGAGC 780

ACCATGCGGA AAAAACATGC TGTCCCCTTC TCTCAGAAGT TCCGCAATAC TGACCCCTTG 840

GCTCTTCGTC TGCTAGAGCG TTTACTGGCA TTTGATCCTA AAGATCGGCC TTCAGCTGAA 900

GAAGCTTTGG CTGATCCGTA CTTCGCAAGT CTTGCTAATG TGGAACGTGA GCCCTCAAGA 960

CATCCAATCT CAAAACTTGA GTTTGAATTC GAGAGACGGA AGCTGACAAA AGATGATGTT 1020

AGAGAATTAA TTTATCGAGA GATTTTGGAG TATCACCCAC AGATGCTGCA AGAGTATATG 1080

AAAGGTGGAG AGCAGATTAG CTTCCTCTAT CCAAGTGGGG TTGATCGCTT CAAACGACAG 1140

TTTGCACACC TTGAGGAGAA CTACAGCAAA GGAGAAAGAG GTTCTCCACT GCAGAGGAAG 1200

CATGCTTCTT TACCGAGGGA GAGAGTAGGT GTATCAAAGG ATGGTTATAA CCAACAAAAC 1260

ACCAATGACC AAGAGAGGAG TGCAGATTCC GTTGCCCGCA CTACAGTAAG CCCTCCAATG 1320

TCACAAGATG CACAACAACA TGGATCTGCT GGCCAAAATG GTGTGACATC CACAGACTTG 1380

AGTTCGAGGA GCTATCTGAA GAGTGCAAGC ATTAGTGCTT CCAAGTGTGT CGCTGTCAAG 1440

GACAATAAAG AACCAGAGGA TGATTACATC TCTGAAGAAA TGGAAGGGTC GGTCGATGGA 1500

TTGTTTGAAC AAGTTTTCAG GATGCAATTC CTAGTGCACA ACGATGACGA TGATCAGTGC 1560

AAGATTTTGT GAGGCGCACC AAATGCTGAT AATTTCCAAG CAGGATGCTG CACTGCAAGT 1620

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GCTCATCCAT GTTCACATAT TCTTCTTGCC ATTGTGCTGT CTGTCACTAC AGGACCCCTG 1740

CATGGATTAA TGTATTATCC CTCTGATGTA ACACTAGATT AGTTCATCTG TCCATGGAGG 1800

AATGAATAGC AAGCAGCCAG CTTGTGCATC ATGTGGGCAT GTTCATTTTC CAGTGAGATC 1860

TAGTCATATC CATGCTTTTT TTGTAATGGT ATATGAAACA GTTTATCAGT GAGACTGTGG 1920

TCCATTCCTC TTTGAAGAAC TCCATTTCCA CTGTGTT 1957

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 519 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Oryza sativa
(B) STRAIN: C101A51

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Glu Phe Phe Thr Glu Tyr Gly Glu Ala Ser Gln Tyr Gln Ile Gln
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Glu Val Ile Gly Lys Gly Ser Tyr Gly Val Val Ala Ala Ala Val Asp
20 25 30

Thr Arg Thr Gly Glu Arg Val Ala Ile Lys Lys Ile Asn Asp Val Phe
35 40 45

Glu His Val Ser Asp Ala Thr Arg Ile Leu Arg Glu Ile Lys Leu Leu
50 55 60

Arg Leu Leu Arg His Pro Asp Ile Ala Glu Ile Lys His Ile Met Leu
65 70 75 80

Pro Pro Ser Arg Arg Glu Phe Gln Asp Ile Tyr Val Val Phe Glu Leu
85 90 95

Met Glu Ser Asp Leu His Gln Val Ile Arg Ala Asn Asp Asp Leu Thr
100 105 110

Pro Glu His Tyr Gln Phe Phe Leu Tyr Gln Leu Leu Arg Ala Leu Lys
115 120 125

Tyr Ile His Ala Ala Asn Val Phe His Arg Asp Leu Lys Pro Lys Asn
130 135 140

Ile Leu Ala Asn Ser Asp Cys Lys Leu Lys Ile Cys Asp Phe Gly Leu
145 150 155 160

Ala Arg Ala Ser Phe Asn Asp Ala Pro Ser Ala Ile Phe Trp Thr Asp
165 170 175

Tyr Val Ala Thr Arg Trp Tyr Arg Ala Pro Glu Leu Cys Gly Ser Phe
180 185 190

Phe Ser Lys Tyr Thr Pro Ala Ile Asp Ile Trp Ser Ile Gly Cys Ile
195 200 205

Phe Ala Glu Leu Leu Thr Gly Arg Pro Leu Phe Pro Gly Lys Asn Val
210 215 220

Val His Gln Leu Asp Ile Ile Thr Asp Leu Leu Gly Thr Pro Ser Ser
225 230 235 240

Glu Thr Leu Ser Arg Ile Arg Asn Glu Lys Ala Arg Arg Tyr Leu Ser
245 250 255

Thr Met Arg Lys Lys His Ala Val Pro Phe Ser Gln Lys Phe Arg Asn
260 265 270

Thr Asp Pro Leu Ala Leu Arg Leu Leu Glu Arg Leu Leu Ala Phe Asp
275 280 285

Pro Lys Asp Arg Pro Ser Ala Glu Glu Ala Leu Ala Asp Pro Tyr Phe
290 295 300

Ala Ser Leu Ala Asn Val Glu Arg Glu Pro Ser Arg His Pro Ile Ser
305 310 315 320

Lys Leu Glu Phe Glu Phe Glu Arg Arg Lys Leu Thr Lys Asp Asp Val
325 330 335

Arg Glu Leu Ile Tyr Arg Glu Ile Leu Glu Tyr His Pro Gln Met Leu
340 345 350

Gln Glu Tyr Met Lys Gly Gly Glu Gln Ile Ser Phe Leu Tyr Pro Ser
355 360 365

Gly Val Asp Arg Phe Lys Arg Gln Phe Ala His Leu Glu Glu Asn Tyr
370 375 380

Ser Lys Gly Glu Arg Gly Ser Pro Leu Gln Arg Lys His Ala Ser Leu
385 390 395 400

Pro Arg Glu Arg Val Gly Val Ser Lys Asp Gly Tyr Asn Gln Gln Asn
405 410 415

Thr Asn Asp Gln Glu Arg Ser Ala Asp Ser Val Ala Arg Thr Thr Val
420 425 430

Ser Pro Pro Met Ser Gln Asp Ala Gln Gln His Gly Ser Ala Gly Gln
435 440 445

Asn Gly Val Thr Ser Thr Asp Leu Ser Ser Arg Ser Tyr Leu Lys Ser
450 455 460

Ala Ser Ile Ser Ala Ser Lys Cys Val Ala Val Lys Asp Asn Lys Glu
465 470 475 480

Pro Glu Asp Asp Tyr Ile Ser Glu Glu Met Glu Gly Ser Val Asp Gly
485 490 495

Leu Phe Glu Gln Val Phe Arg Met Gln Phe Leu Val His Asn Asp Asp
500 505 510

Asp Asp Gln Cys Lys Ile Leu
515

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Synthetic DNA"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AAAAGCACAA GTTGCTGC 18

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Synthetic DNA"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TAACGTCTAT CGACTTCT 18

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Synthetic DNA"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AACACAGTGG AAATGGAGTT CTTCA 25

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1678 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Oryza sativa
(B) STRAIN: C101A51

(vii) IMMEDIATE SOURCE:
(B) CLONE: BIMK1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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CTGCATCCAT CCATCCATCC ATTACTCGCC GAAGACTTCG CGCGGGGAGA GAGGAGGCAA 120

GCTTTGTCGC GGGAACGCGG GAAGAAAGGT CCGAGCTTGG AAGGAGAAGA AGAAGTAGCC 180

AAGAACGCGA GAGCTTGGAA GGCGAGCTGC GGTGGTGTAG CTAGCCAATG CCGGCGGGGA 240

ACTGGTAGGG AGGGGGATGG GGGGAGGGGG CACGCTCGTC GACGGATTCC GCCGCATCTT 300

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GTGAGATCAA GCTCCTTCGT CTGCTCCGTC ACCCAGACAT AGCTGAGATC AAACACATTA 1620

TGCTTCCCCC TTCTCGAAGG GAGTTCCAAG ATATTTATGT TGTTTTTGAG CTCATGGAA 1679

Claims:

1. An isolated deoxyribonucleic acid comprising a nucleic acid sequence that encodes a protein encoded substantially by the sequence from about position 13 through about position 1569 of SEQ ID NO.:1.

2. The deoxyribonucleic acid of claim 1 operably linked to a plant-active promoter.

3. An expression vector capable of transforming a plant cell which contains the deoxyribonucleic acid of claim 1 operably linked to a promoter that is active in said plant.

4. A plant cell transformed with the vector of claim 3.

5. A plant containing a plant cell of claim 4.

6. A seed of the plant of claim 5.

7. The expression vector of claim 3, wherein the plant is a monocot.

8. The plant cell of claim 4, wherein the plant is a monocot.

9. The plant of claim 5, wherein the plant is a monocot.

10. The seed of claim 6, wherein the plant is a monocot.

11. The plant cell of claim 4, wherein the plant is rice, wheat, maize, barley or asparagus.

12. The plant of claim 5, wherein the plant is rice, wheat, maize, barley or asparagus.

13. The plant of claim 5, wherein the plant is rice.

14. A seed of the plant of claim 12 or 13.

15. The deoxyribonucleic acid comprising the sequence from about position 13 through about position 1569 of SEQ. ID. NO.:1.

16. Isolated messenger RNA complementary to the deoxyribonucleic acid of claim 1 or 15.

17. A deoxyribonucleic acid molecule or a ribonucleic acid molecule that hybridizes to the deoxyribonucleic acid of claim 1 or 15 or its complement under stringent hybridization conditions.

18. An isolated protein comprising substantially the amino acid sequence of SEQ ID. NO.:2.

19. A method for conferring disease resistance to a plant which comprises genetically modifying the plant to cause or regulate the expression of the deoxyribonucleic acid of claim 1 or 15.

20. The method of claim 19, wherein the plant is a monocot.

21. The method of claim 19, wherein the plant is rice, wheat, maize, barley or asparagus.

22. The method of claim 21, wherein the plant is rice.

23. A plant promoter having a nucleotide sequence substantially contained in SEQ ID NO:6.

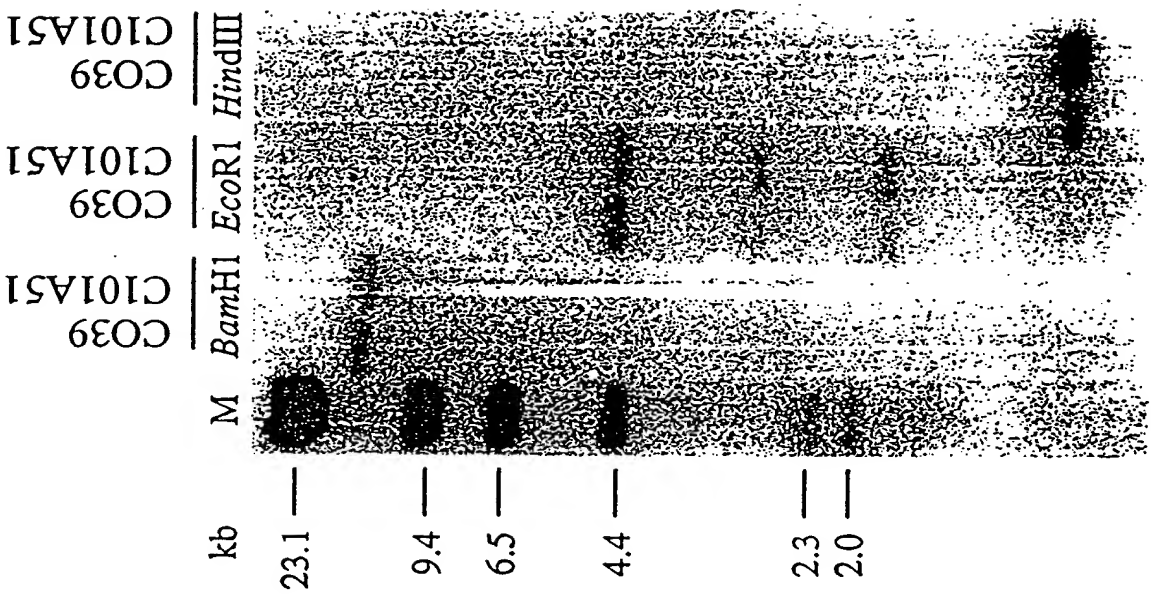

Figure 1

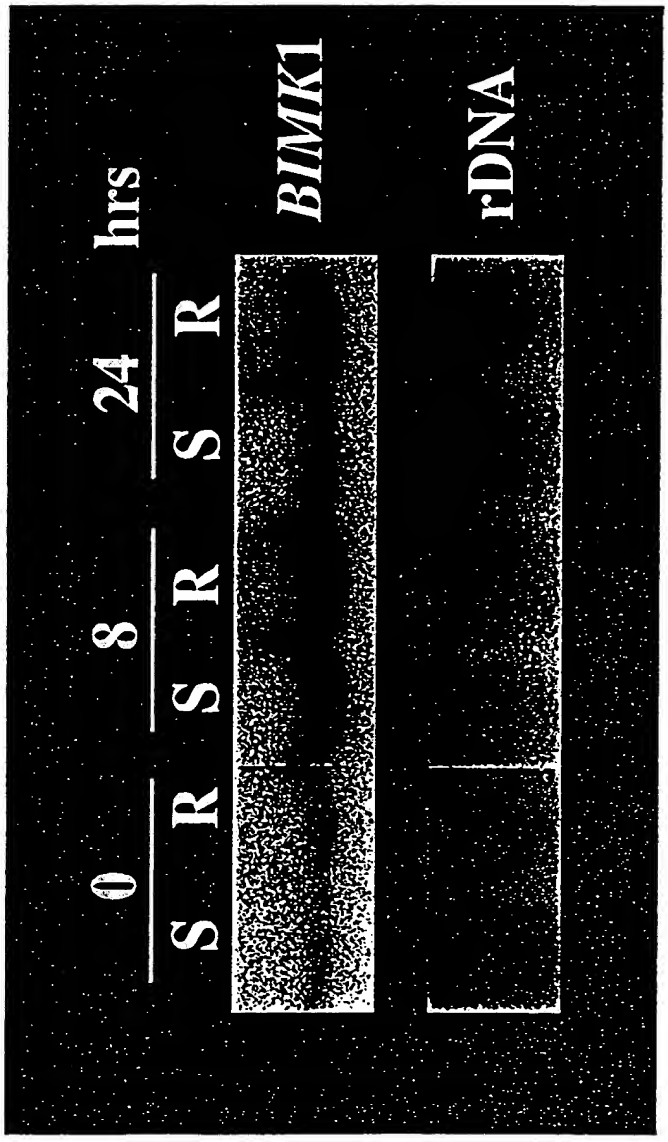

Figure 2

INTERNATIONAL SEARCH REPORT

Interna il Application No
PCT/SG 98/00004

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/29 C12N15/54 C07K14/415 C12N9/12 A01H5/00
A01N65/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A01H A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHOI, D.W., ET AL.: "Selaginella lepidophylla MAP kinase-like protein (sdhn-6r) mRNA, partial cds" EMBL SEQUENCE ACCESSION NO. U96716, 23 May 1997, XP002079536 see the whole document --- -/--	17

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 October 1998	15/10/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Maddox, A

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INTERNATIONAL SEARCH REPORT

Internal Application No
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